Separation of Natural Products Using Thermal Gradients

Introduction

The separation of active components from natural products has become increasingly important for the food, pharmaceutical and nutraceutical industries. Especially in the food and nutraceutical industries, there is increasing pressure to identify both beneficial and potentially harmful ingredients.

This note provides information on the development of high temperature separations for a number of natural products. High temperature liquid chromatography (HTLC) offers fast method development since most separations can be completed using temperature gradients in place of complex solvent gradients. Furthermore, peak shape and peak efficiencies are generally significantly improved at higher temperatures.

Specifically, in this note, we examine the separation of extracts from a number of natural products including cocoa, green tea, and a commercially available ginseng tablet. In these separations we did not attempt to identify all components. The major compounds of interest in this group of products are the catechins (catechin and epicatechin) and caffeine related compounds (caffeine and theobromine) for which chromatographic runs were made and compared against chromatograms for the natural products run under the same conditions.

Analysis Conditions

Instrumentation

All separations invoked the use of the Selerity Technologies Series 8000 HTLC column oven with preheater and effluent cooling. A static pressure regulator maintained a back-pressure on the column of approximately 250 psi. The back-pressure regulator was placed in-line after the effluent had passed through the UV detector cell. The preheater was operated at the same temperature ramp rate as the main oven chamber in all cases.

HPLC pump and ancillary components

The separations described here were done with a variety of HPLC components that are generally commercially available. The Selerity Technologies' Series 8000 HTLC Oven is inserted into existing systems by plumbing the line from the autosampler or manual injector directly through a union to the preheater assembly. From the preheater, the flow passes through the column with the effluent then passing into the UV detector. The preheater and column are located completely within the Series 8000 oven chamber.

Sample preparation

All samples were ground if necessary and extracted in a mixture of methanol/water (80/20). Samples were filtered and loaded into autosampler vials.

Chromatographic conditions

A mixture of water (acidified)/methanol (80/20) was used in all cases. All runs were made under isocratic conditions. Separations were obtained using a Selerity Technologies Blaze C_8 column that withstands temperatures up to 100 °C.

Table 1 :
HTLC Conditions for Analysis of Natural Products

Column:	Selerity Blaze C_8, 100 x 4.6 mm, 3 µm
Mobile Phase:	80:20 Acidic Water:Methanol
Flow:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	Thermal gradient from 40°C to 90°C at 10°/min

Results

Figure 1 (see next page) shows the separation of components in a commercial cocoa preparation (Hershey Company). Overlayed on the chromatogram are the separate chromatograms of catechin and epicatechin, and theobromine and caffeine. The analysis of cocoa is typically done using a solvent gradient of acidified water and methanol.

Figure 2 (see next page) shows the separation of components in a tablet containing ginseng. The tablet was obtained from a nutritional store and is commercially available. The separate chromatogram of theobromine and caffeine is ovelayed onto the tablet chromatogram. The presence of significant peaks corresponding to the retention times of theobromine and caffeine is interesting.

Figure 3 (see next page) shows the separation of components in a green tea preparation. Again the separate chromatograms of catechin and epicatechin, and theobromine and caffeine are ovelayed onto the green tea chromatogram. The analysis of samples such as green tea are typically done using a solvent gradient of acidified water and methanol.

Figure 1: *Cocoa Extract*

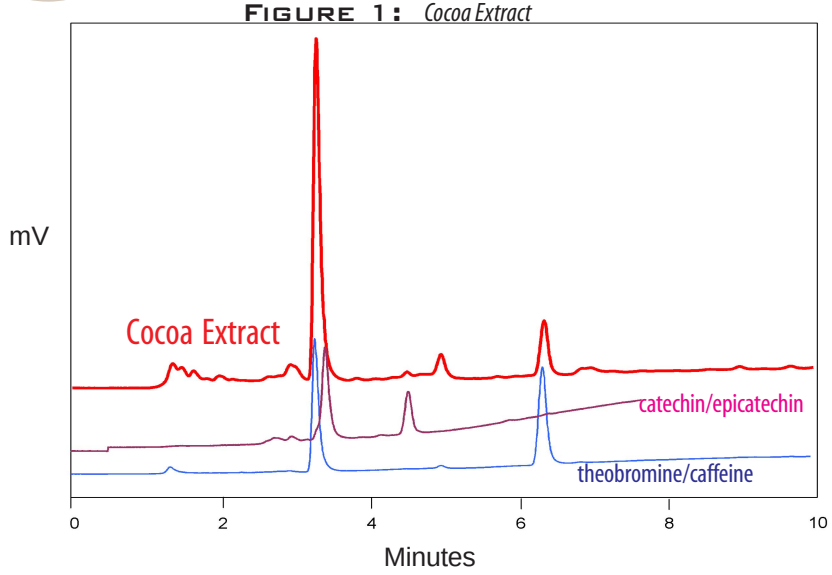


Figure 2: *Gingseng Tablet Extract*

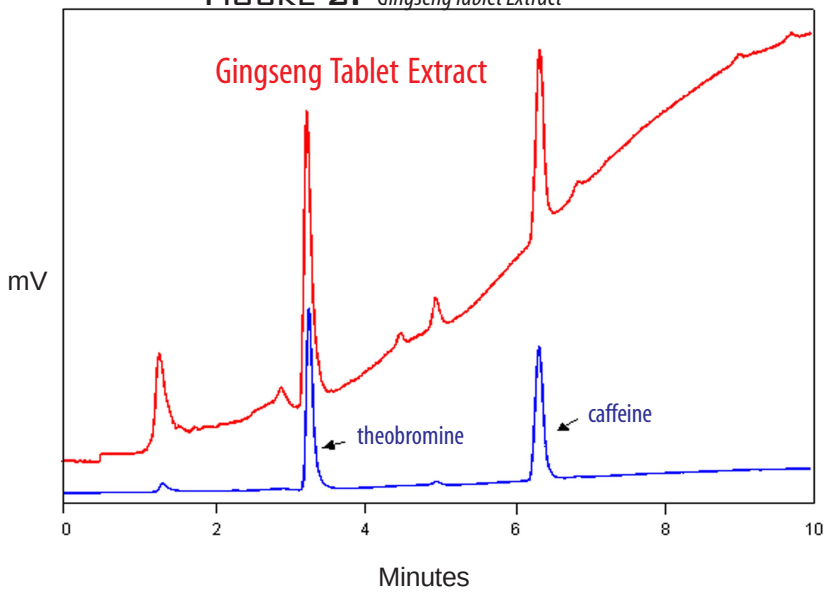
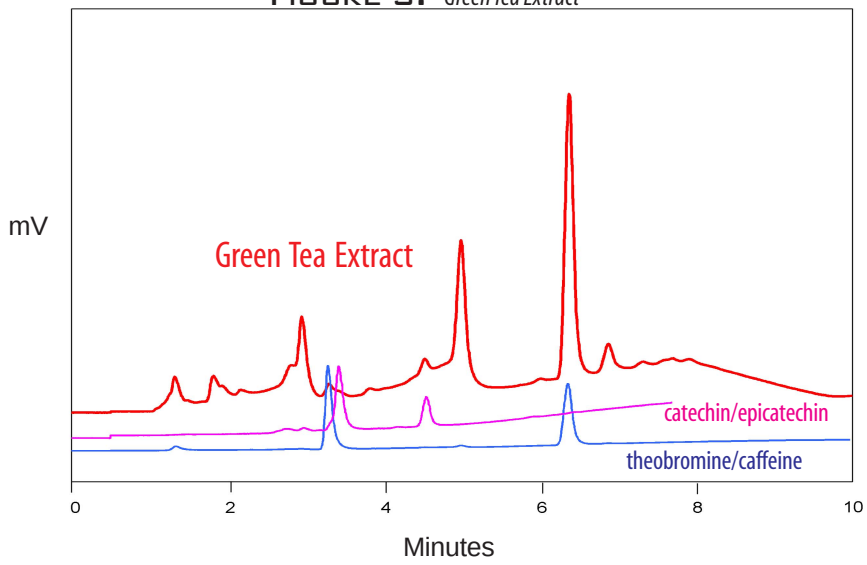


Figure 3: *Green Tea Extract*



Conclusions

Natural products such as those shown here can be conveniently separated using a thermal gradient in place of a solvent gradient program. This has the added advantage that thermal gradients generally result in better peak shape as can be seen in these examples. Method development is rapid. While further improvements in the separations shown could be made, the results shown here were obtained after only two or three preliminary runs.

Acknowledgments

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